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EFFECT OF MGLU1 AND MGLU5 ANTAGONISTS ON MICTURITION REFLEX IN RATS

Hypothesis / aims of study

Ionotropic NMDA and AMPA/kainate receptors are involved in glutamatergic transmission in the micturition reflex pathway and competitive antagonists at these receptors have been found to interfere with the micturition reflex (1).
On the other hand, glutamate also activates a large family of receptors, termed metabotropic glutamate receptors (mGlu). To date, eight subtypes of metabotropic glutamate receptors have been cloned and classified into three groups: Group I (mGlu1 and mGlu5), Group II (mGlu2 and mGlu3) and Group III (mGlu4, mGlu6, mGlu7 and mGlu8). Previous studies (2) reported that i.t. injection of MCPG, a competitive antagonist that does not discriminate between mGlu1 and mGlu5 receptor subtypes, did not change bladder capacity in normal rats. To better evaluate whether the mGlu1 and mGlu5 subtypes are involved in the control of micturition reflex, we examined the effects of selective mGlu1 and mGlu5 allosteric antagonists using different cystometrographic models in anesthetized and conscious rats.

Study design, materials and methods

The selectivity of the tested compounds at mGlu1 and mGlu5 subtypes was evaluated as inhibition of quisqualate-induced Ca^{2+} mobilization in transfected cells expressing rat mGlu1 and human mGlu5 subtypes.
The effects of compounds on the volume-induced rhythmic voiding contractions of the bladder were evaluated in female rats anesthetized with subcutaneous injection of urethane. The bladder was filled via the recording catheter by incremental volumes of saline until spontaneous rhythmic bladder contractions occurred. The activity of compounds was assessed after i.v. administration (through a polyethylene cannula inserted into the jugular vein) in individual animals by measuring the duration of bladder quiescence (disappearance time of bladder contractions; DT) in minutes. The doses inducing 10 min disappearance of contractions (DT_{10min}) were estimated by linear regression analysis.
Cystometry in conscious male rats was performed one day after a catheter (Portex, ID 0.58 mm, OD 0.96 mm) implant in the bladder dome. On the day of the experiment, the rats (fasted overnight) were placed in Bollman's cages; after a stabilization period of 20 min, the free tip of the cannula was connected by a T-shape tube to a pressure transducer and to a peristaltic pump for a continuous infusion of warm saline solution (37°C) into the urinary bladder, at the constant rate of 0.1 ml/min. Two urodynamic parameters from the cystometrogram recorded on the polygraph were evaluated: bladder volume capacity (BVC) and micturition pressure (MP). Basal BVC and MP were evaluated as mean values from the cystometrograms recorded in a 30-60 minute interval prior to treatment. Then the bladder infusion was stopped, the animals were treated orally with the test compound or vehicle and, after restarting bladder filling with saline, changes in BVC and MP were evaluated hourly for 5 hr.

Results

NPS 2407 and R214127 were chosen as selective mGlu1 antagonists, MPEP and MTEP acted as highly selective antagonists for the mGlu5 subtype.
Isovolumic bladder contractions were blocked following intravenous administration of all compounds. However, mGlu1 selective antagonists were less potent than mGlu5 antagonists (Table 1). Furthermore, i.v. administration of R214127 gave a poor dose-response effect.

Tab. 1: Effect of the different compounds tested on VIVC in anesthetized rats after i.v. administration. Data represent the disappearance time (in min, mean ± S.E.) observed after administration of each compound dose and the dose inducing 10 min of bladder quiescence (ED_{10min}).

	mGlu1 allosteric antagonists		mGlu5 allosteric antagonists	
Dose (mg/kg)	R214127	NPS 2407	MPEP	MTEP
0.03	3.7 ± 0.9		3.9 ± 1.4	5.5 ± 1.1
0.1	5.1 ± 1.2	1.7 ± 0.5	7.1 ± 1.0	8.7 ± 2.1
0.3	11.6 ± 1.8	6.4 ± 1.2	12.6 ± 1.7	13.9 ± 2.1
1.0	9.4 ± 2.5	13.2 ± 0.7	22.5 ± 5.3	21.0 ± 4.7
3.0	8.3 ± 2.0	18.2 ± 2.0		
ED_{10min} (95% C.L.)	n.c.	0.562 (0.430÷0.736)	0.150 (0.91÷0.248)	0.106 (0.49÷0.228)

Also in conscious rats, MPEP and MTEP dose-dependently and significantly (after 10 and 3 mg/kg p.o., respectively) increased bladder capacity. Peak micturition pressure was significantly decreased only after administration of MPEP, particularly after administration of the highest dose. Oral administration of NPS 2407 (up to 30 mg/kg) did not induce consistent changes in bladder capacity or micturition pressure.

Interpretation of results

The present pharmacological studies demonstrate that in urethane anesthetized rats the selective mGlu5 allosteric antagonists, MPEP and MTEP, potently inhibit the micturition reflex. NPS 2407, a selective mGlu1 antagonist with potency at mGlu1 similar to that of MTEP at mGlu5 subtype, was significantly less potent than MTEP. After oral administration in conscious rats, MPEP and MTEP were extremely potent at increasing BVC. In contrast, oral administration of NPS 2407 was virtually devoid of effect. The findings given above clearly indicate that a block of mGlu5 receptors has an inhibitory effect on the micturition reflex both in anesthetized and conscious rats. In contrast, mGlu1 inhibition seems to interfere with the micturition reflex only under particular experimental conditions (i.e., isovolumic contractions).

Concluding message

In spite of the promising results obtained in animal models, bladder specific ionotropic glutamate receptor antagonists are not currently available as drugs, most probably because of severe side effects (3). Modulation of synaptic transmission by allosteric antagonists of the mGlu5 glutamate receptor subtype might offer therapeutic benefit in lower urinary tract dysfunctions, such as urge incontinence and overactive bladder.

References

1) Brain Res (1995) 691; 185-194.
2) N. 252, Non-discussion poster at 34th ICS Meeting, Paris, 2004.
3) J Urol (2002) 168; 1897-1913.

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